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Enhanced interleukin 1 generation by monocytes *in vitro* is temporally linked to an early event in the onset or exacerbation of rheumatoid arthritis

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SUMMARY

Twenty-one patients with rheumatoid arthritis (RA) and 12 age and sex matched healthy controls were examined for the ability of their monocytes (adherent cells, AC) to spontaneously secrete interleukin 1 (IL-1) and for their peripheral blood mononuclear cells (PBMC) to secrete interleukin 2 (IL-2) induced by Staphylococcal Protein A (SPA). All RA patients had PBMC which secreted normal amounts of mitogen induced IL-2 regardless of disease activity or disease history. However, AC from RA patients who had a recent (less than 6 months) onset of their disease, or exacerbation of existing RA, had enhanced spontaneous IL-1 secretion. AC from patients with equally active RA but with historically stable disease generated normal amounts of IL-1. Enchanced *in vitro* IL-1 generation by circulating monocytes is temporally linked to an early event in the onset of exacerbation of RA.

Keywords rheumatoid arthritis interleukin 1 interleukin 2

INTRODUCTION

Interleukin 1 (IL-1) and Interleukin 2 (IL-2) are crucial cytokines which drive cell mediated immune responses. Antigen presented in the context of major histocompatibility complex proteins by monocytes, and the monocyte cytokine IL-1, induce IL-2 production by T cells (Smith, Lachman, & Oppenheim, 1980; Palacios & Moller, 1981). Once released, IL-2 promotes the proliferation of IL-2 receptor-bearing T cells regardless of their subclass or antigenic specificity. IL-1 is also necessary for the expression of T cell receptors for IL-2 (Kaye *et al.*, 1984).

Substantial evidence has accumulated suggesting that rheumatoid arthritis (RA) is a disorder of macrophage-T-cell interaction (Janossy *et al.*, 1981). We have previously reported that RA patients with a recent onset or exacerbation of disease exhibited an enhancement of *in vitro* cytokine release (Tan *et al.*, 1984). We were unable to differentiate IL-1 from IL-2 since unseparated peripheral blood mononuclear cells (PBMC) (containing both monocytes and T cells) were used to generate the cytokines. Moreover, the activity was measured with the mouse thymocyte assay, which is generally unable to distinguish between IL-1 and IL-2. We have now re-studied a similar group of patients with either recently active or stable RA using assays specific for IL-1 and IL-2 to determine which cytokine (IL-1, IL-2 or both) was released in excess. Our results show enhanced spontaneous generation of IL-1 by monocytes from recently active RA patients but normal IL-2 generation by their T lymphocytes. RA patients with historically stable disease for more than 6 months had normal *in vitro* generation of both IL-1 and IL-2, regardless of whether their disease was active or inactive at the time of study.

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MATERIALS AND METHODS

Cell preparations. Peripheral blood mononuclear cells (PBMC) were obtained from heparinized blood after separation on Ficoll-Hyqaque (FH) gradients and washed three times with RPMI-1640 (Shore, Dosch & Gelfand, 1979).

IL-1 generation. To obtain monocyte supernatants for the IL-1 determination, 2 ml of 5×10^6 PBMC/ml in 10% fetal calf serum (FCS) (M.A. Bioproducts, Toronto, Canada) and RPMI-1640 + 2 mM L-glutamine + 100 iu/ml penicillin, 100 μg streptomycin, and 0·25 ng/ml Fungizone (PSF) (GIBCO, Grand Island, NY, USA) were incubated in tissue culture dishes (Falcon, Oxnard, CA. no. 3002) for 1 h at 37°C in 5% CO_2. Non-adherent cells were removed by gently washing the dish three times with 2 ml of Hank's Balanced Salt Solution (HBSS). After 2 ml of cold phosphate buffered saline (PBS) was added, the dish was placed on ice for 30 min. Monocytes or adherent cells (AC) were then removed by vigorous pipetting followed by scraping using a rubber policeman and washed in RPMI-1640. Monocyte preparations had less than 5% contaminating T cells by AET-rosetting (Limatibul *et al.*, 1978). Monocytes were cultured for 24 h at 5×10^5 cells/ml in RPMI-1640 supplemented with 10% FCS, L-glutamine and PSF in 5 ml tubes (Falcon, Oxnard, CA, USA, no. 2058). The same batch of RPMI-1640, FCS, HBSS and PBS were used throughout the study. The optimal cell dose and duration of culture for IL-1 generation was determined by kinetic studies. Doubling the number of monocytes did not result in increased IL-1 secretion. The supernatant was harvested, aliquoted and stored at −20°C for assay. In order to assess whether naturally occurring endotoxin had an effect on the spontaneous IL-1 generation, some monocyte cultures were performed in the presence of polymyxin B (PmB) (Burroughs Wellcome Co., NC, USA).

IL-2 generation. Culture conditions for the generation of IL-2 from PBMC of normal individuals were optimized in initial experiments comparing: (a) cell concentrations at 0·5, 1·0, $1{\cdot}5 \times 10^6$ PBMC/ml; (b) mitogens using phytohaemagglutinin (PHA) (Difco, Detroit, MI, USA) at 1/50, 1/100, 1/200, 1/500, 1/1000, 1/1500 final concentrations and Staphylococcal Protein A (SPA) (Pharmacia, Uppsala, Sweden) at 25, 50, 100 μg/ml; (c) serum conditions using 2% and 10% final concentrations of FCS, human AB serum, autologous serum, or no serum, as well as (d) different days of culture (ranging from 1 to 4) (Shore, Leary & Teitel, 1986). IL-2 containing supernatants from PBMC were all subsequently generated under optimal conditions using 10^6 PBMC/ml and 100 μg/ml SPA cultured in quadruplicate cultures of 200 μl microwells (Nunc, Raskilde, Denmark) (2×10^5 cells/well) with serum free RPMI-1640 supplemented with L-glutamine and PSF at 37°C in a humidified 5% CO_2 atmosphere. After a 48 h culture the IL-2 containing supernatants from quadruplicate cultures were pooled, aliquoted, and frozen at −20°C until assayed for IL-2.

In some experiments IL-2 from SPA or PHA induced non-adherent cells (NAC) was measured. NAC were obtained following a single adherence incubation of PBMC (see IL-1 generation) to remove AC. NAC were then washed twice in RPMI-1640 and contained <5% monocytes by esterase stain (α-Naphthyl Acetate Esterase Histozyme Kit No. 90-Al; Sigma, St Louis, MO, USA).

Assay for IL-1. IL-1 was detected by its ability to induce mouse thymocyte proliferation in the presence of a suboptimal dose of concanavalin A (Con A) (Pharmacia, Uppsala, Sweden) (Paetkau *et al.*, 1976). A laboratory standard for the IL-1 assay was prepared culturing purified human monocytes obtained from one donor in the standard way (see 'IL-1 generation'). This internal laboratory standard contained IL-1 activity but had no activity in the IL-2 assay at a 1:2 dilution (see below). Six two-fold serial dilutions of the test supernatants or the internal laboratory standard were added to triplicate 200 μl microcultures of 7–19 week old C3H/HeJ thymocytes (Jackson Labs., Bar Harbor, Maine, USA). Thymocytes were cultured at $3{\cdot}5 \times 10^6$ cells/ml in RPMI-1640 + L-glutamine + PSF with 10% FCS and 0·25 μg/ml Con A. The cell numbers, batch of FCS and mitogen dose were established so as to optimize thymocyte responsiveness while maintaining a low background. After 3 days in culture, cells were pulsed with 1 μCi ^{3}H-thymidine (^{3}H-Tdr) (New England Nuclear Co., Boston, MA, USA) for 6 h, and harvested using a Skatron Cell Harvester (Titertek, Flow Labs, Hamden, CT, USA) with glass fibre filter paper. Counts per minute (ct/min) incorporated were measured using liquid scintillation in a Beckman-7500 counter.

Background ct/min of the Con A stimulated thymocytes were $1{\cdot}8 \pm 0{\cdot}8 \times 10^3$ (mean ± s.d.) in four assays. IL-1 activity of the monocyte supernatants was quantified using a probit analysis of

enhanced ct/min (see below). Our internal laboratory IL-1 standard, arbitrarily assigned 100 units IL-1, generated $51.3 \pm 13{\cdot}4 \times 10^3$ ct/min above background in 4 assays at the 1/40 dilution. A human IL-1 commercial standard, purified using immunoabsorption chromatography by Genzyme, (Boston, MA, USA) and described by them as having 100 units/ml (1 unit = doubling of PHA induced thymocyte proliferation), generated > 100,000 ct/min above background at a 1/640 dilution and > 10,000 ct/min above background at a 1/20,480 dilution in our IL-1 assay. By using our probit analysis, the Genzyme IL-1 had the equivalent of 800 units activity compared with our internal IL-1 laboratory standard. By using the Genzyme definition of IL-1 activity, our mouse thymocyte assay was able to detect > 20,000 units IL-1/ml in their product.

IL-2 assay. IL-2 was assayed using thawed PHA induced T cell blasts previously prepared, aliquoted, and frozen as described (Harel-Bellar, A. *et al.*, 1983). Briefly, human tonsil cells (HTC) were incubated in bulk with 0·1% (1/1000) PHA-P for 48 h, then maintained in PHA stimulated HTC conditioned media until they were no longer responsive to either PHA or SPA. Blasts (usually 18–21 days old) were frozen in liquid nitrogen in 10% DMSO with 20% FCS in aliquots sufficient for the IL-2 assays for one year. For each assay, cells were quick thawed, plated at 2×10^4 cells/well in RPMI-1640 with 2% AB serum, and used to assay IL-2 supernatants in duplicate 72 h cultures. Each supernatant was tested in six two-fold serial dilutions and ct/min above background analysed by probit (see below).

Background ct/min were always less than 1×10^3. An internal laboratory IL-2 standard was pooled from many 1 ml SPA cultures of PBMC from a single normal donor. An external lectin free and chromatographically purified IL-2 standard was obtained from Biotest (Frankfurt/M, FRG). Both standards were used at a 1:4 final concentration plus five serial two-fold dilutions and had reproducible IL-2 activity as assessed by probit analysis in repeated assays (see below). The IL-2 assay could detect up to 1:512 dilution of the commercial IL-2 standard. The 1:4 dilution of the internal laboratory standard generated $43{\cdot}7 \pm 17{\cdot}0 \times 10^3$ ct/min (mean ± s.d.) above background and the 1:4 dilution of the commercial IL-2 standard generated $11{\cdot}4 \pm 6{\cdot}4 \times 10^3$ ct/min above background in eight different IL-2 assays.

Probit analysis of IL-1 and IL-2 data. In order to express IL-1 and IL-2 activity in reproducible, quantitative terms we obtained an activity profile using probit analysis (Gillis *et al.*, 1978). Briefly the ^{3}H-Tdr incorporation data generated from a six $\log_2$ dilution series of the internal laboratory standard was plotted as a percentage of maximum ct/min on probability (probit) paper for each assay (Jordan, 1972). A constant dilution of the standard was arbitrarily defined as 100 units. The x axis dilution coordinate at which the curve of the standard crossed the 50% of the maximum ct/min (Factor_{50}) was determined. Using this procedure the internal laboratory standard for IL-2, the commercial IL-2, and the internal laboratory standard for IL-1 had reproducible activity in repeated assays, thus allowing for comparison between different experiments. For example, the IL-2 commercial standard had a $\log_2 \text{Factor}_{50}$ of $4{\cdot}45 \pm 0{\cdot}5$ (mean ± s.d.) in eight different assays while the internal laboratory IL-2 standard had a $\log_2 \text{Factor}_{50}$ of $7{\cdot}86 \pm 0{\cdot}6$. For the IL-1 assay, the IL-1 internal laboratory standard had a $\log_2 \text{Factor}_{50}$ of $8{\cdot}54 \pm 1{\cdot}7$ in four different assays. The activity of each test supernatant was determined by similarly plotting the ^{3}H-Tdr incorporation of the six-fold $\log_2$ dilution series of each test supernatant on probit paper and comparing the Factor_{50} of each supernatant to the Factor_{50} of the standard.

Patients. Twenty-one patients with classical or definite RA seen at the Wellesley Hospital, University of Toronto Rheumatic Disease Unit were studied. Patients had not received, more than 7·5 mg per day corticosteroids or other immunosuppressives within 3 months of the study. Results from these patients were compared with 12 age and sex matched healthy controls. Patients were divided into two major groups: (1) those with stable disease (active or inactive) unchanged for more than 6 months and (2) those with a recent (within 6 months) disease onset or exacerbation of disease, defined as a greater than two-fold increase in the number of actively inflamed joints. Patients in the latter group, with the exception of one patient, had their onset or exacerbation of disease within 3 months of testing. Patients with stable RA, i.e. group 1, comprised two subsets: (i) patients with active disease arbitrarily defined as having > 5 actively inflamed joints and (ii) patients with inactive disease (all of whom had one or fewer inflamed joints and no generalized morning stiffness).

Statistics. Statistical analyses were carried out using the Mann-Whitney U2-tailed test and results expressed as mean ± s.d.

RESULTS

As seen in Table 1, the patients with active but historically stable disease exhibited a degree of joint inflammation comparable to the patients with a recent onset or exacerbation of their RA. Both of these groups differed markedly from the group of patients with stable inactive disease in terms of variables of disease activity.

In the course of 12 months, the purified monocytes from 31 different normal individuals had a spontaneous IL-1 generation of 51·2 ± 34·9 units. This was similar to the IL-1 generation by monocytes from 12 normal subjects (age and sex matched to the RA patients) used for the purpose of this study (43·0 ± 31·1 units) ($P > 0·05$). The use of cells from at least one normal subject with each experiment assurred a control for day to day variability in our assay over time. Monocytes obtained from RA patients who had stable arthritis generated similar amounts of spontaneous IL-1 ($n = 13$, 39·8 ± 14·5 units) ($P > 0·05$). Noteably, spontaneous IL-1 generation by monocytes from patients with stable active disease (subset i) did not differ from that generated by monocytes from patients with stable inactive disease (subset ii) ($n = 6$, 37·0 ± 11·0 *v* n = 7, 43·1 ± 19·0 units IL-1 respectively, $P > 0·05$). In contrast, IL-1 generation by monocytes from RA patients with a recent onset or exacerbation of their disease activity showed a markedly enhanced ability to spontaneously generate IL-1 ($n = 8$, 115·6 ± 41·5) ($P < 0·01$) (Fig. 1).

Endotoxin may be present in laboratory materials and may induce IL-1 production (Dinarello, 1984). Polymyxin B (PmB), a cationic antibiotic, prevents endotoxin from inducing IL-1 both *in*

Table 1. Clinical information on patients with rheumatoid arthritis*

		Stable arthritis for > 6 months	
	Recent onset or flare ($n = 8$)	Active arthritis ($n = 7$)	Inactive arthritis ($n = 6$)
Age	49 ± 20·1	52·5 ± 13·0	58·2 ± 22·5
M/F	2/6	3/4	2/4
Disease duration (years)	9·6 ± 6·5	7·5 ± 6·8	13·8 ± 11·1
Functional class	2·4 ± 0·5	2·3 ± 0·8	1·3 ± 0·4
Morning stiffness (min)	162·5 ± 148	111·0 ± 84·6	0
No. active joints†	19·5 ± 9·9	15·6 ± 7·9	0·3 ± 0·5
No. effusions	8·3 ± 3·6	6·2 ± 4·9	0
Landsbury index	61·1 ± 33·8	62·3 ± 50·2	5·3 ± 9·7
Damage/duration index‡	0·66 ± 0·64	0·92 ± 0·8	0·20 ± 0·49
ESR (mm/h)	52·5 ± 14·8	30·0 ± 11·4	16·8 ± 16·6
Platelet count	376·5 ± 96·2	282·8 ± 117·7	261·3 ± 42·6
Rheumatoid factor	634 ± 1336	606·3 ± 698·8	77·5 ± 73·9
Remittive agents§	gold-1 pt pen-1 pt	nil	chlor-2pt. gold-2 pt. pen-1 pt.

* All data expressed as mean ± s.d.

† Defined as number of joints with either stress pain or an effusion.

‡ Defined as number of damaged joints (determined by either clinical examination, or erosions, subluxation, or ankylosis on radiographs) divided by the duration of disease in years.

§ Chlor chloroquin; pen penicillamine.

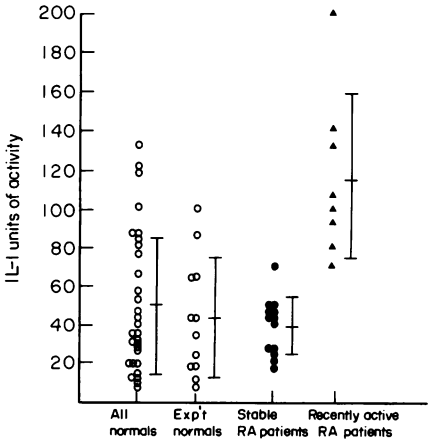

Fig. 1. IL-1 units of activity measured from monocyte cultures of normals, experimental age and sex matched controls and RA patients. Mean ± s.d. is depicted.

vivo and *in vitro* (Duff & Atkins, 1982; van Miert & van Duin, 1978). Including PmB in cell cultures at 5–10 μg/ml effectively prevents endotoxin-stimulated IL-1 production without impairing cell function (Ikejima *et al.*, 1984). In order to determine if naturally occurring endotoxin was responsible for generating spontaneous IL-1 by monocytes, various concentrations of PmB were added to monocyte cell suspensions at the initiation of culture. IL-1 supernatants were then tested for their activity in the mouse thymocyte assay as before. Controls consisted of PmB at similar concentrations added to the IL-1 supernatant at the time of the assay. PmB at the relevant concentrations added before or after the generation of the IL-1 supernatants had no effect on IL-1 generation as measured by mouse thymocyte proliferation (Table 2). The results indicate that

Table 2. Effects of PmB on spontaneous IL-1 generation

Concentration of PmB added (μg/ml)*	^{3}H-TdR incorporation by C3H/HeJ thymocytes (ct/min × 10^3)†
nil	21·5
PmB added at initiation of monocyte culture	
0·5	24·5
5·0	33·0
50·0	22·5
PmB added to supernatant at end of monocyte culture	
0·5	21·0
5·0	32·5
50·0	21·0

* Monocytes were cultured for 24 h in RPMI-1640 + 10% AB serum with or without PmB.

† Mean of two separate 72 h cultures of Con A stimulated $3{\cdot}5 \times 10^6$ thymocytes/ml in 0·2 ml microwells with a 1:20 dilution of supernatant. Background ct/min was $1{\cdot}15 \pm 0{\cdot}34 \times 10^3$ (mean ± s.d. of 24 microcultures).

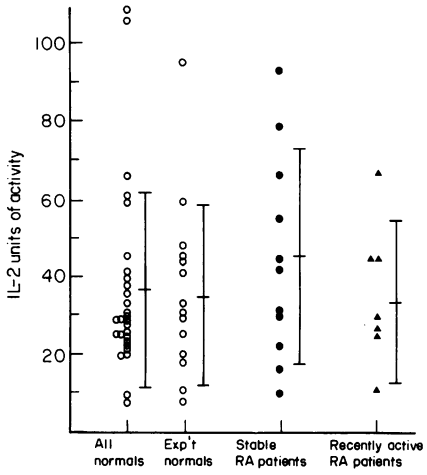

Fig. 2. SPA induced IL-2 units of activity measured from PBMC cultures of normals, experimental age and sex matched controls and RA patients. Mean ± s.d. is depicted.

naturally occurring endotoxin did not contaminate our procedures for IL-1 generation and is not responsible for the IL-1 generation in our cultures.

The ability of unseparated PBMC to generate IL-2 following exposure to the mitogen SPA was also studied in the same subjects simultaneously with the IL-1 generating cultures. In marked contrast to the enhanced spontaneous IL-1 production by isolated monocytes from recently active RA patients, IL-2 generation by their SPA stimulated PBMC was similar to both normals and RA patients with stable disease (Fig. 2).

In the course of 18 months, the PBMC from 27 normal individuals had an SPA induced IL-2 production of 37·4 ± 24·8 units. The PBMC from 14 age and sex matched normal controls used for the purpose of this study, had a similar SPA mediated IL-2 generation of 36·2 ± 22·4 units. IL-2 production by SPA induced PBMC cultures from seven subjects with recently active RA was 35·0 ± 17·9 units ($P > 0·05$ relative to controls). PBMC from 11 subjects with stable RA generated 44·2 ± 26·4 units IL-2 ($P > 0·05$ relative to controls). PBMC from RA patients with stable disease generated similar amounts ($P > 0·05$) of IL-2 whether or not their disease was active (subset i; 50·5 ± 34·2, units $n = 4$) or inactive (subset ii; 42·7 ± 16·7 units, $n = 7$) at the time of study.

To determine if our failure to detect mitogen induced IL-2 abnormalities was due to our use of SPA, we cultured the PBMC from six normals and six subjects with active RA with both SPA or PHA simultaneously in separate cultures. Confirming our earlier kineties, 100 μg SPA/ml was

Table 3. IL-2 generation induced by PHA or SPA in PBMC from RA patients and normals*

Subjects	PHA†	SPA‡
Rheumatoid arthritis ($n = 6$)	19·8 ± 6·9	79·2 ± 11·6
Normals ($n = 6$)	27·2 ± 18·1	56·7 ± 31·0

* Cultures of 10^6 PBMC/ml in 200 μl microwells with serum free RPMI for 48 h. IL-2 assay as described in text and expressed as units activity (mean ± s.d.).

† PHA 1:1000 dilution.

‡ SPA 100 μg/ml.

Table 4. IL-2 generation by PBMC and NAC*

		Subjects			
		Normals		RA	
Mitogen	Expt.	PBMC	NAC	PBMC	NAC
PHA 1:1000	1	0·9	0·5	0·5	10·5
	2	0·6	2·7	9·0	42·0
	3	3·6	2·7	16·5	33·4
	4	2·1	2·8	1·6	4·3
	5	1·8	1·7	4·5	4·9
	6	ND	ND	3·6	9·6
	7	ND	ND	5·3	7·3
SPA 100 μg/ml	8	6·2	6·6	9·1	9·1
	9	4·0	4·8	9·2	7·9
	10	8·4	10·1	10·2	13·9

* IL-2 expressed as the average ct/min $\times 10^3$ of duplicate T-blast cultures minus background (always $< 1{\cdot}0 \times 10^3$ ct/min) for 1:4 dilution of supernatant.
nd Not done.

superior to any dilution of PHA from 1:100 to 1:1000 in inducing IL-2 generation. PHA induced IL-2 production was somewhat lower in PBMC from RA patients compared with normals ($19{\cdot}8 \pm 6$ units versus $27{\cdot}2 \pm 18{\cdot}1$ units. However, in neither the SPA nor the PHA cultures were PBMC from RA subjects significantly different from normals in their ability to generate IL-2 ($P > 0{\cdot}05$ relative to normals) (Table 3).

We also compared the ability of PHA and SPA to induce IL-2 in cultures of PBMC and monocyte diminished PBMC (non-adherent cells, (NAC)) from normals and patients with RA. NAC cultures from normals and RA patients secreted the same amount of IL-2 using SPA as a mitogen. In contrast, if PHA was used as a mitogen, NAC produced at least two-fold more IL-2 than PBMC in 5/7 experiments using cells from RA patients. Monocyte depletion had no effect on PHA induced IL-2 from normal PBMC (Table 4).

DISCUSSION

Our results demonstrated an enhanced ability of monocytes from recently active RA patients to spontaneously generate IL-1. These patients all had the onset or an acute exacerbation of their disease as defined in our previous work (Tan *et al.*, 1984) i.e. within 6 months; but in 7/8 this had occurred within 3 months. The PBMC from these patients however generated normal amounts of IL-2 compared with age and sex matched controls. These results are consistent with our earlier finding of enhanced generation of cytokines by the PBMC from a group of recently active RA patients which could promote proliferation of Con A stimulated mouse thymocytes (Tan *et al.*, 1984). In our original study we also found PBMC from such patients also had enhanced proliferation to exogenous IL-2 alone without mitogen, indicating increased number of T cells with IL-2 receptors or increased numbers of IL-2 receptors on each T cell. Furthermore, there was a close correlation between cytokine production and IL-2 responsiveness. The current findings of enhanced IL-1 but normal IL-2 generation is consistent with these phenomena since IL-1 promotes the expression of IL-2 receptors rather than just promoting IL-2 synthesis (Kaye *et al.*, 1984). Our results also concur with the finding that rats with adjuvant-induced arthritis have peritoneal macrophages which produce higher amounts of IL-1 (Gilman *et al.*, 1984). It is not surprising that

the enhanced IL-1 generation by monocytes from our RA patients did not lead to enhanced IL-2 production by their PBMC. The generation of IL-2 by SPA-induced PBMC cultures could not be enhanced even by the addition of a 1:4 dilution of commercial IL-1, thus demonstrating that under the *in vitro* experimental conditions, factors other than IL-1 limit IL-2 generation.

The detection of clear-cut spontaneous IL-1 from unstimulated monocyte cultures may be due to the sensitivity of our IL-1 assay (see 'Materials and Methods'). Spontaneous IL-1 production by monocytes has been previously described (Lachman & Metzgar, 1980) and may be due to trauma during isolation procedures (Dinarello, 1984). IL-1 activity also occurs spontaneously without stimulation following the culture of epidermal cells (Sauder *et al.*, 1982), and human alveolar macrophages from normals (Koretzky *et al.*, 1983). Endotoxin contamination was not a likely factor in the spontaneous IL-1 release of our culture system since similar amounts of IL-1 were secreted by monocytes even in the presence of PmB. It is possible that FCS contains factors other than endotoxin which may promote IL-1 release by monocytes (Hoffman, Mizel & Kirst, 1984). It is relevant that we were careful to use the same batch, not only of FCS, but of all media used throughout this study.

Enhanced spontaneous generation of IL-1 in our subgroup of recently active RA patients suggests *in vivo* activation of their monocytes. Monocytes from RA patients are also known to have increased numbers of Fc receptors (Moller-Rasmussen *et al.*, 1982), increased complement synthesis (De Ceulaer *et al.*, 1980) and elevated activity of their hexose monophosphate shunt (Kay *et al.*, 1979), all features indicative of activation.

IL-1 is a likely candidate capable of mediating much of the local joint pathology in RA. The mononuclear cell factor (MCF) which stimulates RA synovial cells to produce collagenase and PGE_2 has been found to be IL-1 (Mizel *et al.*, 1981). IL-1 production within joints can thus mediate the degradation of cartilage, and via PGE_2, bone resorption (Robinson, Tashjihan & Lavine, 1975). IL-1 also is known to affect cultured human chondrocytes causing them to produce plasminogen activator as well as PGE_2 (McGuire-Goldring *et al.*, 1984). IL-1 has been detected in RA joint effusions but not in effusions from patients with Osteoarthritis (OA) by Fontana *et al.* (1982). In contrast, Nouri, Panayi and Goodman (1984) and Wood *et al.* (1983), both detected IL-1 in non-inflammatory effusions from patients with OA as well as in effusions from RA.

Our data concerning IL-2 conflicts with a recent report by Combe *et al.* (1985), demonstrating decreased IL-2 generation by PHA stimulated PBMC from RA patients. This difference may be due to our use of SPA rather than PHA for IL-2 generation. We and others have found that IL-2 generation by PBMC in response to PHA is highly variable and that up to 25% of normal individuals have very low responses (Redelman, Lisse & Zvaifler, 1983). PHA induced IL-2 production is greatly influenced by the presence of monocytes, which if present in excess, decrease the amount of IL-2 generated by PBMC cultures (Redelman *et al.*, 1983; Shore *et al.*, 1986). When monocytes exceed 10% of cultured cells there is suppression of PHA induced IL-2 generation (up to 75% suppression) which is partially indomethacin sensitive and is in part, but not wholly mediated by prostaglandin synthesis (Palacios, 1982). In contrast, during 18 months of study we found that in only six of 148 separate experiments using SPA pulsed PBMC from normal individuals did cultures fail to secrete IL-2. We have also found that SPA induced IL-2 production PBMC is not inhibited even when culture conditions are modified so that 33% of cells are monocytes (Shore *et al.*, 1986).

The variability in monocyte proportions in normal individuals may explain why PHA, but not SPA, induced IL-2 generation is so inconsistent in PBMC from normal subjects. However, Table 4 demonstrates that monocyte suppression of IL-2 generation may be a more frequent problem in RA PBMC than PBMC from normals. In the 10 normal subjects tested neither SPA nor PHA induced IL-2 generation by PBMC was influenced by monocyte depletion. However, monocyte depletion did influence PHA but not SPA induced IL-2 generation by PBMC from five of seven RA patients (Table 4). The use of soluble SPA as a mitogen for the stimulation of IL-2 production was first used by Fleischer (1981). Other than its lack of susceptibility to monocyte inhibition, there are several theoretical reasons for using SPA to induce IL-2. It has been found to be a potent T cell mitogen (Romagnani *et al.*, 1984; Schuurman, Gelfand & Dosch, 1980); SPA may preferentially affect T helper cells (Lipsky, 1980) and studies in both humans and mice indicate that the IL-2 producer may have a T helper cell phenotype (Wagner & Rollinghoff, 1978; Palacios & Moller, 1981).

By elucidating an early systemic event during the evolution of RA, attractive avenues of further speculation and investigation may have been identified. Since IL-1 production is enhanced only transiently early in RA, it probably does not play a pathogenetic role in the perpetuation of RA. Further work needs to determine whether enhanced IL-1 has a casual or causal relationship to the infective or inflammatory process(es) initiating RA. This mode of study is, however, able to differentiate the function of circulating mononuclear cells from RA and those from systemic lupus erythematosus (SLE). PBMC and monocytes from SLE, in contrast to RA, have been shown to produce decreased amounts of both IL-1 and IL-2 (Linker-Israeli *et al.*, 1983).

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